

## Deposition and Simultaneous Concentration of Dilute Solutions in Paper Partition Chromatography

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In adapting partition chromatography to the identification of histamine in extracts of biological materials, it became evident that the deposition of the customary 0.01 to 0.1 ml volume of fluid to the paper strips would not provide adequate amounts of histamine. The concentrations of histamine encountered were too low relative to the sensitivity of the color reaction employed as indicator

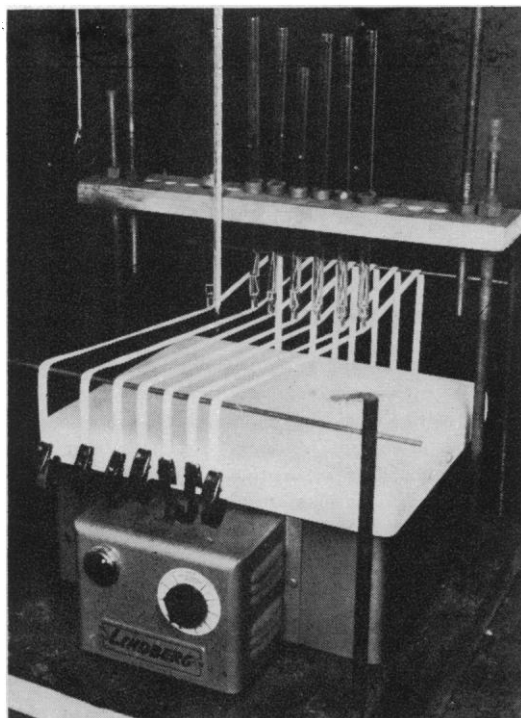


FIG. 1.

on the developed chromatogram. Therefore, it became necessary to deposit the entire final extracts, consisting of 3–5 ml of butanol, without allowing excessive spreading of the solvent on the strips.

Various procedures were tried, such as application of the solutions from very fine capillaries, pipettes plugged with cotton, and pipettes with sintered glass tips with and without simultaneous drying of the solvent with heat and by air blast. Since uniform delivery could not be obtained by any of these methods, the following procedure was finally chosen as most satisfactory.

The paper strips, cut to the proper length and marked at the point of application, are stretched horizontally

above a hot plate. Both ends of the strips are weighted by means of window display hooks. Capillary pipettes are brought into contact with the application points marked on the strips. The pipettes are inserted through holes in a rack and held by means of rubber sleeves cut from pressure tubing (Fig. 1). The solutions to be

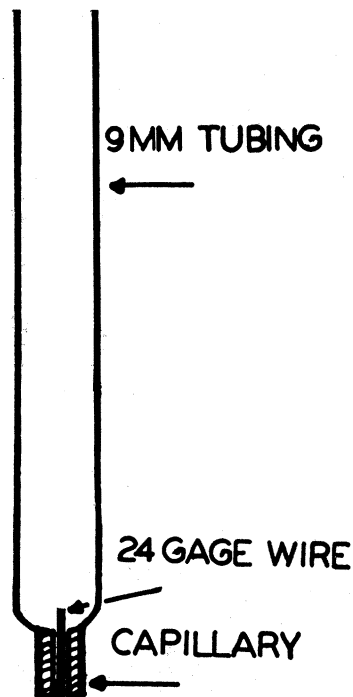


FIG. 2.

applied to the paper strips are transferred to the pipettes when the temperature at the level of the strips has reached the desired point. For our particular purpose, the pipettes are constructed of 9-mm Pyrex tubing, approximately 20 cm long. To one end an 8-mm length of capillary tubing is fused. The bore of the capillary is of such caliber that it will just admit a 10–12-mm length of 24-gage stainless steel or platinum wire (Fig. 2).<sup>1</sup> With this arrangement, approximately 1 ml/hr of butanol is delivered to and evaporated from the strips at a temperature of 60–70°. The assembly shown in Fig. 1 allows the simultaneous application of solutions to 14 strips with a minimum of attention. The wet spots on the strips spread no more than 2–3 cm at any time. The rate of delivery can be decreased by making the capillary longer or by using less liquid in the wide part of the pipette. After use, the wires are removed from the capillaries by pushing them out with a glass rod inserted through the 9-mm tubing, and the pipettes cleaned by letting them stand in alkaline cleaning solution.

Other organic solvents, such as ether, acetone, alcohol, dioxane, etc., can be applied in a similar manner. Aque-

<sup>1</sup> The pipettes were kindly suggested and made by J. J. Svarz, Department of Chemistry, Northwestern University Dental School.

ous solutions tend to spread excessively on the paper. Solutions containing even weak hydrochloric or sulfuric acid char a variety of the papers tried, even at moderately elevated temperatures. The method may prove useful in other applications of partition chromatography where the concentration of the substance to be identified is low in relation to the sensitivity of the color or other indicator reactions.

## A Simple Device For Exposure of Groups of Mice to Uniform X-Ray Doses<sup>1</sup>

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In the course of experiments involving exposure of groups of mice to doses of X-rays<sup>2</sup> it was necessary to utilize a holder which assured uniformity of exposure. Based on a metal "pie plate" device of Snyder (1, 2), this device was constructed to minimize the possible errors in dosage due to reflection of X-rays and interference in passage through the metal screen cover.

Circles of  $\frac{1}{4}$ "-thick plywood,  $5\frac{1}{2}$ " and  $7\frac{1}{2}$ " in diameter, were cut and enclosed in a 2"-wide strip of  $\frac{1}{4}$ "-mesh wire hardware cloth, so that the wooden plaque was mounted in the middle of the wire cloth band. A cover of plastic screen mesh, ordinarily used in window screening, was made by stretching a piece of the plastic mesh across an embroidery hoop of the proper diameter. (See Fig. 1.)

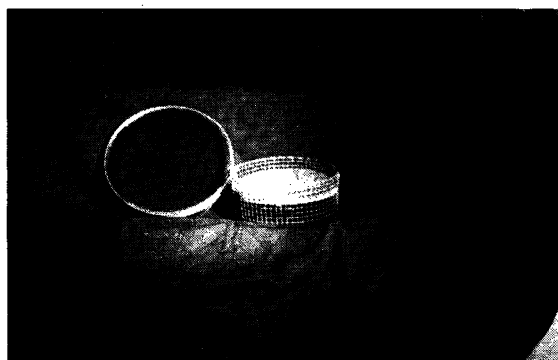


FIG. 1.

In such a device the X-rays do not pass through any metal before reaching the animals, thereby avoiding adulteration of the beam with secondary radiations. The plywood "floor" of the device is elevated 1" above the table; thus there is a minimum of back scatter. Twenty-four mice can be simultaneously irradiated in the  $7\frac{1}{2}$ " tray and ten to fourteen mice in the  $5\frac{1}{2}$ " tray.

### References

1. LIE, P. Y., SNYDER, J. C., and ENDERS, J. F. *J. exp. Med.*, 1941, 73, 699.
2. Personal communication.

<sup>1</sup> Designed in connection with radiation study under Atomic Energy Commission and Office of Naval Research contract.

<sup>2</sup> By Milton Friedman, radiologist, New York University.

## Infrared Spectrometry in Metabolic Studies With Deuterium-labeled Steroids<sup>1</sup>

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Infrared spectrometry, which has been of the greatest utility for the detection, identification, and isolation of steroid metabolites excreted in urine (2, 3, 6), has been extended to the identification of steroids with deuterium in the molecule. Changes in the infrared absorption spectra as a result of the replacement of hydrogen by deuterium were noted first by Hardy, Barker, and Denison (4) in 1932. The analysis of deuterium-containing organic compounds of biological interest by means of infrared spectrometry has since been described by Herget and Hardy (5). Our investigations have been concerned with the detection of a deuterium steroid in an extract containing a number of closely related compounds. One marked advantage of the spectroscopic technique, especially in physiological studies, is that the material to be examined can be recovered without loss or alteration. It is thus possible to employ deuterium as a tracer where a pure compound can be obtained only in limited amount.

In Fig. 1 the spectrum of pregnanol-3( $\alpha$ )-one-20 is compared with the spectrum of the same compound where a hydrogen atom at C-11 and at C-12 has been replaced by deuterium. Two absorption bands attributed to the C-D stretching vibrations appear at 2,165 and 2,145  $\text{cm}^{-1}$  in the deuterium-containing compound. In the neighborhood of 1,200  $\text{cm}^{-1}$  pronounced differences are apparent in the two spectra. These latter differences can be ascribed to the perturbing effect of the increased mass of the deuterium atoms on the molecular vibrations. In general, with an increase of the number of deuterium atoms in a steroid there is a progressively greater change in the spectrum between 700 and 1,300  $\text{cm}^{-1}$ , as well as an increase in intensity of the bands at 2,165 and 2,145  $\text{cm}^{-1}$ . These observations are expected since it has been shown that the spectrum in the region 700–1,300  $\text{cm}^{-1}$  is extremely sensitive to any change in structure (3).

The C-D absorption bands in the neighborhood of 2,150  $\text{cm}^{-1}$  are particularly useful for the identification of a deuterium-containing molecule because, in the concentrations used, this region is transparent in the absence of

<sup>1</sup> Aided by grants from the American Cancer Society (on recommendation of the Committee on Growth of the National Research Council), Ayerst, McKenna and Harrison, Ltd., the Jane Coffin Childs Memorial Fund for Medical Research, the Commonwealth Fund, the Anna Fuller Fund, the Lillia Babbitt Hyde Foundation, the Albert and Mary Lasker Foundation, the National Cancer Institute of the National Institute of Health, U. S. Public Health Service (grants C822 and C440) and the New York Foundation.